

Antifungal drug resistance pattern of *Candida. spp* isolated from vaginitis in Ilam-Iran during 2013-2014

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Abstract:

Vaginal Candidiasis is the most common and important opportunistic fungal infection in women. By increasing use of antifungal drugs in recent years, it has caused drug resistance. This study aims to evaluate antifungal drugs susceptibility of *Candida. spp* isolated of women with vaginitis from Ilam-Iran during 2013-2014. samples were collected and cultured from 385 women with vaginitis, then *Candida.spp* was diagnosed by standard method. Antifungal drug susceptibility test for nystatin 100 unit/disk, fluconazole 10µg/disk, itraconazole 10µg/disk, ketoconazole 10µg/disk, amphotericinB 20µg/disk, clotrimazole 10µg/disk, posaconazole 5µg/disk, and voriconazole 1µg/disk were carried out by M44-A method(CLSI). From all culture positive samples, 150 isolates were *Candida albicans* and 89 isolates were non-*albicans*. The resistance to fluconazole, itraconazole, ketoconazole, clotrimazole, voriconazole, posaconazole, nystatin and amphotericin B was 76%, 62%, 72%, 55%, 6%, 7%, 1% and 0%. The highest resistance was seen for fluconazole, itraconazole, and the highest susceptible was seen for nystatin and amphotericin B. These results indicate nystatin and amphotericin B can be used as the first line for empirical therapy of vaginal candidiasis in the district.

Key words: Drug resistance, *Candida albicans*, vaginitis.

Background:

Nowadays, fungal infections are increasing especially in immune-deficiency patients [1-5]. There is a common problem with vaginitis [6] caused almost by both *Candida* species and *Gardenerlla vaginalis* as well as *Trichomonas vaginalis*, which are responsible for 10 millions official visits in a year [7-11]. The infection of Candidal vulvovaginitis is originated from lower genital found in 30-80 % of cases without symptoms or mild symptoms [12]. Almost 70 % of women have experienced the vulvovaginal Candidiasis at least once [13-15]. About 45% of women have suffered from 2 or more attack in a year, and about 5% of them have suffered from chronic and recurrent infection [16-17]. The most common sign of Candidal vaginitis is vaginal itching, dysuria and malodorous-white vaginal

secretions [18, 19, 20]. In fact, the candidal vulvovaginitis especially in recurrent type is a physical-mental disease which causes a lot of stress in patients [21] that is rarely a threat agent, but it led to catastrophic results such as recurrent suffering, more consumption and sterility. According to a research in U.S, every recurrent of this disease spent 60 \$ for visit and medical treatment and a doctor spent 1-3 hours for diagnosis and treatment [22]. Some important factors which cause Candidiasis to increase are pregnancy, contraception drugs with high estrogen, antibiotic consumptions, uncontrolled diabetes, immuno-suppressive drugs, unsafe or excessive sexual intercourse, chronic anemia and season allergic [23-26]. For treating Candidal vaginitis, the most available way is using local azol family such as clotrimazole [27]. The most abundant

use of nystatin is the treatment of facial infections such as mucosal candidiasis including vaginal or gastrointestinal infections [28]. Other members of topical azol family are butoconazole, miconazole and terconazole. The fluconazole and ketoconazole are used orally and has the same effect [27-29]. However, resistance to azol in *Candida* species has been increasing in recent years and has caused serious problems in successful treatment of vaginitis. The resistance to azole drugs in non-albicans species such as *C. glabrata*, *C. parapsilopsis*, *C. tropicalis*, *C. krusei* is increasing [30-32]. Regarding resistance increase to antifungal drugs in *Candida* species, the present study evaluated antifungal drug susceptibility of fungi isolated from vaginitis of women referred to privacy clinic of Ilam city during 2013-2014.

Methodology:

Sampling and culture

Samples were collected from 385 women with vaginitis by obstetrician by using sterile swab after collecting, the samples were transported to laboratory and were cultured in Sabouraud Dextrose Agar containing chloramphenicol 0.5% and were incubated in 37°C for 2 days. Differentiation of *C. albicans* and non-albicans isolates, were carried out by germ tube test, chlamydospores producing, growth on chrome agar and carbohydrate absorption [33].

Antifungal drug susceptibility test

The reference MM4-A method suggested by Clinical and Laboratory Standards Institute (CLSI) was used for antifungal drug susceptibility test [34]. *C. albicans* and non-albicans isolates were cultured overnight in 35°C, then a suspension equal 0.5 McFarland was prepared, and was cultured on Mueller Hinton agar containing 2% glucose and 0.5 mg/ml methylene blue, by using sterile cotton swab. Antifungal drug disks were used including nystatin 100 unit/disk, fluconazole 10µg/disk, itraconazole 10µg/disk, ketoconazole 10µg/disk, amphotericin B 20µg/disk, clotrimazole 10µg/disk, posaconazole 5µg/disk, and voriconazole 1µg/disk (Sigma Aldrich, USA). The inhibition zone diameters were recorded after 24 h and 48 hours [35]. The *C. albicans* ATCC 2091 was used as standard strain.

Statically analysis

All of data were analyzed by SPSS v.18, chi-square and T-student test and $P < 0.05$ was considered as significant.

Results:

Among 385 samples, culture of only 239 samples was positive, and 150 isolates were *C. albicans* and 89 isolates were non-albicans. Among 385 samples, the 133 samples from urban persons and the 225 from rural persons were collected. The 61 urban samples were *C. albicans* ($P < 0.05$). Eighty three women of all patients were 25-35 years old and 22 persons were less than 25 years old, and 45 persons were more than 35 years old, and 32.5% of them had history of using antifungal-drug **Table 1** (see supplementary material). This study showed that the patients who were *C. albicans*-positive culture, about 33 persons had used condoms, 22 persons in normal way, 73 persons had used high estrogen contraceptive (more than 40mg/µg) and 22 persons had used fallopian tube obstruction method and other ways for contraception ($P < 0.05$). There was 6 illiterate persons, 26 persons with BS degree, 2 persons with MSc degree, 48

persons with diploma degree and 68 persons with under-diploma degree ($p < 0.05$). In the antifungal drug susceptibility test, resistance to 8 antifungal drugs including fluconazole, itraconazole, ketoconazole, clotrimazole, voriconazole, posaconazole, nystatine, amphotericin B were 76%, 62%, 72%, 55%, 6%, 7%, 1% and 0%, respectively **Table 2** (see supplementary material).

Discussion:

Researchers are interested in antifungal drug resistance because of increasing fungal diseases in predisposed people such as those suffering from AIDS, diabetic persons, those who use broad spectrum antibiotics and chemotherapy, and also, growing drug resistance in fungi. In this study, about 150 isolates from all 239 isolates were *C. albicans* and 89 isolates were non-albicans, that it showed the high prevalence role of *C. albicans* in candidal vulvovaginitis [36]. There is a significant relation between urban and rural disposed persons to vulvovaginitis, and it is in accordance with Mohamadi *et al*; [37]. Also, the results showed the high prevalence of candidal vulvovaginitis in 25-35 years old and it is in agreement with the results of Gharibi *et al*; [38]. There is a significant relation between the way of contraception and candidal vulvovaginitis ($P = 0.05$), that is, the most abundant pollution which had been seen in women who used high estrogen contraceptive drugs, in fact it occurred because of changing in hormones level and pH level in vaginal, subsequently [39]. In the study of Kotarski *et al*; that was done among the polish women in Art state, it showed that candidal vulvovaginitis is the common reason for referring to gynecological in sexual active persons, and it is in line with our results [40, 41]. There is a significant relation between the academic degree of participant and the chance of suffering from candidal vulvovaginitis, according to the results, we can claim that higher education has direct relation with higher level of personal hygiene, that it was in line with Azizi *et al*; and Abdollahi *et al*; [42, 43]. In the present study, the resistance of *C. albicans* isolated from vaginitis to 8 antifungal agents indicate, the highest resistance was seen for fluconazole with 79% and the lowest was seen for nystatin and amphotericin B with 1% and 0%, respectively. In the present study, the resistance of nystatine and amphotericin B that they originated from poly-N family and caspofungin from echinocandin family, was less expected, while in the other study that performed on the resistance of *C. albicans*, it was reported 3-8% [44-48]. This result indicates the low resistance of *C. albicans* isolated from Ilami patients, it may be due to using herbal antifungal medicine in this city [49]. The resistance to fluconazole with 79% was more than the other studies [50, 51]. The resistance to voriconazole and posaconazole were similar to other studies, but there was high unexpected resistance reported about itraconazole, ketoconazole and clotrimazole comparison Mohamadi *et al*; that performed in 2014 and evaluated the resistance of *C. albicans* isolated from Candidiasis suspected neonates, this difference in results may be related to different age groups and samples [52].

Conclusion:

The results of this study indicate high susceptibility to nystatin and it is recommended to use this antifungal drug in empirical therapy for candidial vaginitis. Regarding high resistance to fluconazole, itraconazole and ketoconazole, these antifungal drugs are not recommended for treating candidial vaginitis.

Ethical Considerations:

This research project was approved by the ethics committee of Ilam University of Medical sciences and name and Specifications of patient were kept confidential and Samples from patients was with Personal satisfaction.

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Supplementary material:

Table 1: History use of antifungal agents in person with candidiasis

Took antifungal	Candida albicans	
Yes	Frequency	49
	Percent	32.5
	Total	49
No	Frequency	101
	Percent	67.5
	Total	101
Total	Frequency	150
	Percent	100
	Total	150

Table 2: Frequency of resistance and susceptibility to antifungal drugs in C.albicans isolated from vaginitis

		flu	itr	ket	clot	vori	posa	nys	amph B
C.albicans	Susceptibility % (frequency)	53	69	37	89	143	138	145	150
	Dose-dependent % (frequency)	18	23	41	6	1	5	4	0
	Resistance % (frequency)	79	68	72	55	6	7	1	0